Primary ciliary dyskinesia in the genomics age

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Abstract: 191/150-200

Primary ciliary dyskinesia (PCD) is a genetically and clinically heterogeneous syndrome. Impaired function of motile cilia causes failure of mucociliary clearance. Patients typically present with neonatal respiratory distress of unknown cause, and then continue to have a daily wet cough, recurrent chest infections, perennial rhinosinusitis, otitis media with effusion and bronchiectasis. Approximately 50% of patients have *situs inversus,* and male and female infertility is common. Whilst understanding of the underlying genetics and disease mechanisms have substantially advanced in recent years, there remains a paucity of evidence for treatment. Next generation sequencing has increased the trajectory of gene discovery, and mutations in >40 genes have been reported to cause PCD, with many others likely to be discovered. Increased knowledge of cilia genes is challenging our perceptions of the clinical phenotype, as some recently reported genes are associated with more mild respiratory disease. Developments in genomics and molecular medicine are rapidly improving the diagnosis of patients, and a genetic cause can be identified in approximately 70% of patients known to have PCD. A number of groups are now investigating novel and personalized treatments, although gene therapies are unlikely to be available in the near future.

Key messages

- Primary ciliary dyskinesia (PCD) is a syndrome caused by mutations in genes responsible for structure and function of motile cilia;although mutations have been identified in more than 40 responsible genes, the genetic cause of PCD cannot yet be found in ≈25% of patients. .
- A PCD diagnosis can be confirmed by identification of a hallmark ultrastructural defect by transmission electron microscopy or bi-allelic pathogenic mutations in a known PCD gene.
- Genetic advances are identifying patients with atypical presentations of PCD and have confirmed some overlaps with non-motile ciliopathies
- No diagnostic test is perfect for PCD; therefore, diagnosis relies on a combination of tests and no test can be used in isolation.
- A number of genotype-phenotype associations have been described in small studies, and international collaboration and prospective registries are now needed to properly understand these.
- In the absence of disease-specific evidence, respiratory management of PCD is based on evidence from cystic fibrosis. PCD guidelines recommend regular airway clearance and treatment of pulmonary exacerbations with antibiotics for all patients. Early steps for personalised medicine are being taken in light of genetic understanding.

Introduction:

Primary ciliary dyskinesia (PCD) is a rare syndrome, characterized by extensive genetic heterogeneity and clinical variability. Mutations in over forty genes have been reported to cause PCD, and genes continue to be discovered. Abnormal ciliary function leads to unexpected neonatal respiratory distress in term infants, persistent wet cough from early infancy, bronchiectasis, chronic rhinosinusitis, and conductive hearing impairment; 50% of patients have situs inversus, and infertility is common [1]. Disease progression is highly variable, with some patients maintaining reasonably good lung function and quality of life into later adulthood, whilst others have worse outcomes[2-5]. There is increasing evidence that mutations in different genes lead to variable phenotypes, for example some genes are never associated with situs anomalies, and variants in others are more likely to cause infertility [6,7]. There is limited but increasing evidence that some genes are associated with severity of pulmonary disease[8-11].

PCD is estimated to affect 1:10-15,000 Europeans, and is more common in populations with closed genetic pools[12-14]; interestingly genetic heterogeneity is seen in socially isolated consanguineous populations[15,16]. Estimates of prevalence are limited outside Europe, but it is expected that PCD is more common in certain populations, such as those from Arabic countries [17,18]. A survey of European PCD specialists reported that only a small percentage of the expected patients have been diagnosed, and patients reported through an international survey that 37% had visited a doctor with PCD-related symptoms more than 40 times before being referred for testing[12,19]. The reasons for under-diagnosis are multi-factorial including difficulty accessing diagnostic services and the lack of awareness of the syndrome amongst general physicians[19]. Symptoms are non-specific, and patients with situs inversus, which is rare in the general population, are diagnosed earlier than those with normal organ positioning[12]. Clinical tools have been developed to help physicians identify patients for testing[20,21], but these are based on 'typical' symptoms. We are increasingly aware of genotypes which are associated with atypical presentations.

As with other rare diseases, the evidence base for treating patients is lacking. In addition to a handful of small PCD studies, consensus guidelines are based on evidence from more common disorders such as cystic fibrosis (CF) and chronic rhinosinusitis[22,23].

In this review, we discuss the current state-of-the-art regarding the underlying mechanisms of PCD and how this might inform our understanding of clinical presentation and natural disease progression. We review current diagnostic and management strategies for patients with PCD. We will discuss how the improving knowledge of PCD genetics is impacting our understanding of the

phenotype, our conduct of diagnostic testing and our management of patients. The authors reflect on the expanding phenotype, and how this challenges our definition of PCD.

Search strategy and selection criteria

This manuscript is not a systematic review but consists of the authors' expert knowledge of the disease area informed by the literature; we searched PubMed, for evidence relating to PCD in the English language since 2000: using the search term "primary ciliary dyskinesia" in combination with the following: (diagnosis; clinical symptoms/clinical presentation/phenotype; treatment/clinical trial). After identifying eligible studies, we checked for additional citations in their reference lists.

Clinical features

Patients with PCD usually present with a classic clinical phenotype[1,20,21,24]. Defining and understanding these manifestations allows for earlier recognition and identification of these patients. In PCD, over eighty percent of the population will present with respiratory distress at birth despite being term. This distress typically occurs 12 to 24 hours after birth and many infants require prolonged oxygen due to hypoxemia[25]. Furthermore, chest radiographs in infants with PCD often reveal lobar collapse.

Impaired mucociliary clearance in this population leads to chronic wet cough associated with rhinorrhea. These manifestations occur daily and typically begin during the first months of life. The majority of patients with PCD have recurrent acute otitis media, otitis media with effusion, and chronic otitis media[1,26,27]. Repeat ear infections may lead to transient or permanent hearing loss, the latter causing significant morbidity later in life. Sinusitis is a common manifestation in this population, but may not be detected in younger patients due to lack of imaging[8,23].

Recurrent pulmonary infections due to impaired mucociliary clearance ultimately leads to bronchiectasis. Respiratory cultures (sputum, bronchoalveolar lavage) in the younger population often reveal *Streptococcus pneumonia*, *Moraxella catarrhalis*, *Staphylococcus aureus* and *Hemophilus influenzae*. *Pseudomonas aeruginosa* may be detected in children with PCD, but becomes more predominant in adults[8,28-30]. Chest computed tomography imaging during infancy have revealed early airway manifestations, including bronchiectasis[31]. Infertility is often present in males. In females, fertility may be difficult due to impaired cilia motion in the fallopian tubes[7,24].

Situs inversus occurs in fifty percent of PCD patients; and situs ambiguus, including heterotaxy, has been reported in up to 12% of patients with PCD[6,32,33]. These patients have an increased incidence of

congenital heart disease. Children and adults with situs inversus and/or heterotaxy associated with respiratory manifestations should be evaluated for PCD[23,34].

Diagnosing PCD has historically been difficult due to the need for specialized testing coupled with lack of general awareness leading to the failure of clinicians to recognize the classic phenotype. Recently, four clinical features were defined as predictive of PCD, including laterality defect; unexplained neonatal respiratory distress; early-onset, year-round nasal congestion and wet cough. The sensitivity and specificity of having PCD if all four features were present was reported at 0.21 and 0.99, respectively[21]. In another study, a scoring tool, PICADAR (Primary Ciliary Dyskinesia Rule) was developed to predict the diagnosis of PCD in a symptomatic population[20]. The seven features in addition to persistent wet cough that were predictive of PCD included full-term gestation, neonatal chest symptoms, admission to the neonatal intensive care unit, chronic rhinitis, ear symptoms, situs inversus and congenital cardiac defect. PICADAR represents a diagnostic algorithm that may prove useful when delineating whether a work-up is indicated for PCD (score of 4: sensitivity 0.97, specificity 0.48; score of 5: sensitivity 0.90, specificity 0.75 [20]. However, these predictive diagnostic tools may be under-utilized if general awareness remains low. Efforts to raise knowledge, led by specialists in the field, is already improving referrals. In the UK, the age of diagnosis in the pediatric population has dropped from 4.8 years (2009)[12] to 2.6 years (2015- unpublished data). In North America, there are now 41 accredited PCD Foundation centers; this initiative is increasing the identification and recognition of this rare disease. Further research is warranted in high risk populations to investigate whether predictive tools with targeted nasal nitric oxide testing could further promote early referral. Adult bronchiectasis clinics are a likely reservoir of undiagnosed patients, and work is needed to raise awareness amongst adult pulmonologists. Modified predictive tools will be needed for this population since many adults are unlikely to recall early life events which are captured by the current questionnaires[20,21]. For example a modified PICADAR score and low nasal nitric oxide was accurate in identifying PCD amongst adults with bronchiectasis[35].

Significant heterogeneity exists within the PCD population in age-matched lung function values[2,3,8]. In some cases, genotype accounts for the variability between patients, for example mutations in *CCDC39* and *CCDC40* are associated with worse lung function, whilst *DNAH9* mutations are associated with milder disease[8-10]. In a recent publication, the rate of FEV_1 decline was highly variable in 137 patients with PCD followed at seven centers for five years[9]. Furthermore, ventilation inhomogeneity as measured through the multiple breath washout technique may be abnormal even with normal spirometric values[36,37].

Diagnosis

Multiple investigations are usually required to make a diagnosis of PCD. There are two evidence based diagnostic guidelines published by the European Respiratory Society (ERS) and the American Thoracic Society (ATS) respectively[34,38]. Both guidelines recognise that there is no perfect diagnostic test for PCD. There is a lack of sensitivity or specificity for most tests used, specialist equipment and expertise is required and consequently access to testing can be problematic. Guidelines recommend a combinations of the following tests:

Nasal Nitric Oxide (NO)

The level of NO gas in the nasal cavity can be measured with a gas analyser. This test is ordinarily reserved for children who have developed paranasal sinuses and are able to comply with velum closure manoeuvres (usually >4yrs old). Most individuals with PCD have NO values which are tenfold lower than healthy controls[39]. Sensitivity and specificity are good (98% and >99.9%, respectively) at a cut off at 77nl/min when measured in patients with symptoms of PCD, with a chemiluminescent analyser, using ATS/ERS NO measurement guidelines[40]. However there can be several other causes of reduced nasal NO such as smoking or nasal obstruction. Patients with cystic fibrosis are also reported to have reduced NO values[41,42]. Conversely, although a normal nasal NO level remains helpful to exclude a diagnosis, there are exceptions where nasal NO is above the typical cut-off used for diagnosing PCD. These include individuals with mutations in *GAS8*, STK36, *CCDC103, RSPH1* and *GAS2L2*[11,43-46]. Increasing knowledge of genetics has highlighted that nNO cannot be used in isolation as a screening test, as certain patients with PCD will be missed.

New portable analysers make NO testing increasingly accessible in the clinic, although both sensitivity and specificity when using these analysers is reduced[47].

High speed video microscopy analysis (HSVMA)

Live samples, usually obtained by nasal brushing, can be assessed for ciliary beat frequency and pattern. This functional assessment is achieved ex-vivo using a light microscope fitted with a high speed video camera. Ordinarily cilia beat in a metachronal wave with an effective forward stroke followed by a recovery stroke. Specific beat patterns are observed in patients with PCD: completely immotile, immotile with occasional residual movement, reduced bend and reduced beat amplitude, hyperfrequent with reduced beat amplitude or with a circular motion when viewed from above. In some cases a mixed phenotype can occur. Correlations between cilia beat pattern and ultrastructural defect or genotype in PCD have been demonstrated[48,49]. Ciliary beat frequency is less accurate when used in isolation and is dependent on several factors, such as patient age, formulation of the maintenance media and sample temperature[50]. Recent evidence shows excellent

accuracy of HSVMA for diagnosis, however the limitation is the specialist equipment required to record the videos and the expertise required to interpret the beat pattern. Also pathogenic variants in some genes can be associated with fairly normal ciliary beating (e.g. *RSPH1, CCDC103, DNAH9)*

Immunofluorescence:

Antibodies directed against ciliary proteins are becoming increasingly available and are used regularly in a research setting to confirm absence of ciliary protein and the pathogenicity of a genetic mutation[51]. Recent evidence for the use of antibody testing with a panel of six antibodies shows similar sensitivity and specificity as TEM in clinical practice[52].

Transmission electron microscopy:

Normal ciliary ultrastructure consists of a "9+2" structure: 9 peripheral microtubule doublets, surround the central pair of single microtubules (Figure 1). Each doublet is connected with the central pair by radial spokes, and neighbouring doublets are joined by the nexin dynein regulatory complex (N-DRC). The outer microtubular doublets contain regularly repeating structures known as outer and inner dynein arms (ODA and IDA), which are responsible for the generation of ciliary motion through ATPase activity.

Since the first description of the condition by Afzelius, PCD diagnosis has relied on ultrastructural analysis by TEM[53]. TEM remains a robust test to confirm a diagnosis although increasingly cases with normal ultrastructure have been identified [50,54,55]. Despite poor sensitivity, the specificity of TEM for diagnosis of PCD is excellent[34]. As noted above, normal ciliary ultrastructure may occur in those with PCD. Hallmark diagnostic defects for PCD include: absence of the ODA, absence of the ODA and IDA, and absence of the IDA with microtubular disorganisation. In addition ultrastructural defects which can support a diagnosis when used in combination with other tests have been identified. These include central complex defects, mislocalisation of basal bodies, isolated microtubular disorganisation and partial defects of the outer +/- inner dynein arms affecting <50% of cross sections. Figure 2 shows examples of the various ciliary ultrastructural changes according to PCD genotype.

Genotyping

Both ERS and ATS guidelines agree that a bi-allelic pathogenic mutation or hemizygous X-linked mutation in a known PCD gene confirm a diagnosis[34,38]. Around 65-70% of PCD cases diagnosed by other methods can be genetically solved. However many individuals have variants of unknown

significance or mono-allelic heterozygous variants which require confirmation by functional and structural tests (HSVMA, IF and TEM). It is not unusual to identify one or several rare missense variants that are not linked to the disease, due to the large size and number of genes sequenced in PCD patients; therefore there is significant risk of false positive results unless the genetic diagnoses are supported by corresponding HSVMA or TEM results (Table 1). Since genetic testing fails to identify ≈30% of patients, it cannot exclude a diagnosis. nNO with hallmark TEM or repeatedly abnormal HSVMA can probably identify most patients with PCD associated with normal genetics to ensure they receive appropriate clinical care.

Summary

We emphasise the risks of relying on any one test to diagnose PCD, and the benefit of using a combination of tests[50]. No test is perfect, and it is only by comparing results from various tests that the clinician can be confident. Importantly, all diagnostic tests should be conducted an interpreted by PCD experts who understand the relative risks of false positive and negative results for the individual tests. Due to imperfect testing by all modalities it is likely that some patients with PCD are not recognised despite adherence to ATS or ERS guidelines. Therefore research and development in PCD gene discovery and improved diagnostic techniques are crucial. Novel approaches such as radiolabelled mucociliary clearance provide additional data which may help exclude a diagnosis of PCD when this functional test is normal in difficult diagnostic cases [56,57]. In addition new advances in electron microscopy such as 3D electron tomography provide insight into PCD cases previously thought to have normal ultrastructure [58].

Genetics and disease mechanisms

Mutations of more than 40 different genes have been reported that cause PCD (Figure 1). Most PCD variants are transmitted in an autosomal recessive fashion. However, X chromosomal recessive inheritance has also been reported for a few genes[59,60]. Motility of respiratory cilia is generated and regulated by complex mechanisms involving several large multimeric protein complexes. Mutations affecting many of these proteins can result in PCD. In approximately 50% of cases, mutations in some genes are associated with laterality defects, whilst mutations in other genes are always associated with situs solitus. Dyskinesia of the cilia severely impairs mucocilary clearance, leading to recurrent and chronic airway infection and inflammation. IL-8, neutrophils, and neutrophil elastase activity are significantly elevated in the PCD airway, contributing to the inflammatory environment[61,62]. Furthermore, peripheral blood monocytes from PCD patients produce higher levels of inflammatory cytokines when stimulated compared to those from healthy individuals, perhaps

contributing to the chronic airway inflammation and resulting tissue damage from infections [63]. This recurrence and persistence of airway infections and inflammation contribute to progressive lung disease and eventually bronchiectasis.

PCD associated with laterality defects

PCD individuals with abnormal outer dynein arm (ODA) composure, abnormal ODA docking machinery, abnormal cytoplasmic dynein preassembly or tubular disorganization share the possibility during embryogenesis of randomization of left-right asymmetry. Thus, half of all affected individuals exhibit situs inversus or heterotaxy, because those disease mechanisms also affect nodal cilia function important for determination of left-right asymmetry during early embryogenesis. The association of situs inversus with PCD is also referred to as Kartagener syndrome.

Defects of outer dynein arm composure: The ODA, attached to the outer doublets, generate the main mechanical force to produce cilia bending. Genetic defects in genes that encode ODA components such as the heavy chains DNAH5, DNAH11[64,65], the intermediate chains DNAI1 and DNAI2[66,67] as well as light chains such as DNAL1 and NME8[68,69] all result in dysfunction of ODAs.

Mutations in the gene *DNAH5* result in the most frequent defect reported in PCD individuals. High-speed video microscopy analyses (HSVMA) of cilia beating reveals immotile cilia; transmission electron microscopy (TEM) and immunofluorescence microscopy analysis (IF) confirm deficient/ absent ODAs.

Mutations in genes encoding intermediate chains of the ODAs such as DNAI1 and DNAI2, or encoding the light chain DNAL1 cause similar defects to *DNAH5* mutations that can be recognized by TEM, IF and HSVMA. Interestingly, mutations in the *NME8* gene have been reported to result in a variable phenotype of the ultrastructure[69]. Approximately half of the cross sections showed ODA defects, the other half showed normal composure. In the original reports it had been suggested that some cilia might lack ODAs and that other cilia might assemble ODAs. However, recently it has been discovered that two distinct ODA populations are present in respiratory cilia along the ciliary lengths. ODA complexes type 1 containing the beta-heavy chain DNAH11 are located in the proximal compartment whereas ODA complexes type 2 containing the beta-heavy chain DNAH9 are located in the distal compartment[10,51,70]. Thus, it is more likely that in NME8 mutant cilia the distal ciliary axonemes lack ODAs type 2.

DNAH11 mutations cause a distinct PCD phenotype characterized by a hyperkinetic beating pattern with reduced beating amplitude. The bending of the proximal ciliary compartment is deficient in *DNAH11* mutant cilia. *DNAH11* mutations do not cause obvious ultrastructural defects readily

identifiable by TEM[55]. Therefore, *DNAH11* mutations are often overlooked in centres that rely only on TEM to establish PCD diagnosis. Immunofluorecence microscopy with anti DNAH11 antibodies can detect some PCD individuals with mutations in *DNAH11*[71]. Recently mutations in *DNAH9* have been reported in PCD individuals with mild or no respiratory disease[70]. *DNAH9* mutant cilia lack ODAs of the distal ciliary axonemes and exhibit only subtle beating abnormalities[10,70].

Immunofluorescence microscopy analysis utilizing antibodies directed against ODA components DNAH9, DNAH11, DNAH5, DNAI1 or DNAI2 can easily depict the distinct defects of ODA composure in the different genetic PCD variants.

Defects in outer dynein arm docking and targeting: ODAs are attached to the outer doublets by complicated mechanisms involving a multimeric docking machinery which are referred to as ODA docking complexes (ODA-DCs). Mutations in genes encoding different ODA-DC components such as CCDC114[14,72], ARMC4[73], CCDC151[74,75] and TTC25 [76] can result in abnormal ODA docking. Thus, mutations of these genes result in absence of ODAs from the ciliary axonemes, usually easy to detect by TEM and IF. HSVMA readily identifies predominantly static cilia. In addition, with the use of antibodies targeting the ODA docking machinery it is possible to distinguish molecular defects caused by abnormal ODA composure or by abnormal ODA docking. Interestingly, the ODA-DC protein TTC25 appears to be the major component of the ODA docking machinery because in those mutant cells, ODA-DCs such as CCDC114, CCDC151 and ARMC4 cannot be assembled. In contrast, *ARMC4* mutations cause abnormal ODA docking that is more prominent in the distal ciliary axoneme than proximally resulting in a heterogeneous picture along the axoneme. CCDC103 [43,77] is not a classical ODA-DC protein but is involved in ODA targeting. *LRRC56* mutations disrupt intra-flagella transport-dependent delivery of ODA components and result in subtle defects of distal ODAs[78].

Defects in cytoplasmic preassembly of dynein arms: ODAs as well as inner dynein arms (IDAs) which are important for the regulation of the ciliary beating are preassembled in the cytoplasm prior to delivery to their final docking sites at the outer doublets along the ciliary axoneme. Therefore, mutations in genes that alter cytoplasmic preassembly processes result in defects of ODAs as well as IDAs. Those preassembly factors are referred to as dynein axonemal assembly factors (DNAAFs). Mutations in genes that encode these cytoplasmic preassembly factors such as DNAAF1 [79,80], DNAAF2[81], DNAAF3 [82], DNAAF4(DYX1C1)[83], DNAAF5 (HEATR2)[84], LRRC6 [85], ZMYND10 [86,87], SPAG1 [88], C21ORF59 [89], DNAAF6 (PIH1D3) [59,60] and CFAP300 (C11ORF70)[90] cause PCD with combined ODA and IDA defects. Because the cilia lack outer as well as IDAs, most mutations result in complete cilia immotility, easily identified by HSVMA. For *DNAAF2* as well as for hypomorphic mutations in

DNAAF4, some residual cilia motility can be retained due to partial assembly of outer and IDAs. The defects are usually more pronounced in the distal ciliary axoneme than proximally.

In male individuals carrying mutations in genes encoding cytoplasmic preassembly factors, sperm dysmotility indicates that the molecular disease mechanisms of cytoplasmic preassembly also plays a role in sperm flagella.

Defects of the ninety six nanometer ruler machinery: The ninety six nanometer ruler proteins CCDC39 [91] and CCDC40 [92] are responsible for the correct establishment of the 96 nm repeats along the ciliary axoneme. In each 96 nm axonemal repetitive unit four ODAs and various distinct IDAs are attached to the outer doublets. Those ruler proteins are also important for anchoring the dynein regulatory complex (nexin links) connecting the outer doublets as well as IDA proteins such as DNALI1 to the axoneme. The cilia beat with a reduced bend and amplitude. The ultrastructural defects seen by TEM comprises a severe tubular disorganization as well as deficiency of IDA components; the ODA composure is not affected. In all reported cases, recessive mutations in the genes *CCDC39* or *CCDC40* have been identified. With the use of IF it has been confirmed that mutant cilia lack nexin link proteins such as CCDC164 [93], CCDC65 [89], GAS8[45,94] or LRRC48. In addition, patients lack IDA proteins like DNALI1 in the axonemes. Thus, HSVMA, TEM and IF can easily identify patients with defects in the genes *CCDC39* or *CCDC40*.

PCD without laterality defects

Several disease mechanisms can alter cilia motility of multiciliated respiratory cells whilst not affecting function of nodal monocilia during early embryogenesis. Those genetic defects are not easily diagnosed by TEM and they can benefit from analysis by IF.

Defects in radial spoke components: Mutations in the genes encoding radial spoke proteins *RSPH1* [95]*, RSPH4A* [96]*, RSPH9* [96]*, RSPH3* [97] and *DNAJB13* [98] cause abnormal radial spoke composure. However, this abnormal radial spoke composure causes only subtle ciliary beating abnormalities, usually comprising of rotational or stiff beating patterns. IF shows abnormal composure by demonstrating absence of radial spoke head proteins[99].

Defects in central pair associated proteins : Genetic defects encoding central pair associated proteins such as HYDIN [100] and STK36 [46] have been reported. Those genetic defects are not associated with obvious ultrastructural abnormalities. In some cross sections occasionally central pair abnormalities can be noted by TEM. However, those abnormalities can also occur in normal individuals and they are not pathognomonic. Genetic testing for *HYDIN* mutations is complicated. Due to a recent evolutionary event, *HYDIN* became duplicated and therefore most of the coding

exons of *HYDIN* are also present in the pseudogene *HYDIN2*. Due to this duplication DNA sequence analysis of *HYDIN* is not easily established. Therefore, some commercial genetic kits do not screen for *HYDIN* mutations or report a reduced sensitivity for this genetic testing. Diagnosis of central pair defects is also hampered by the fact that some individuals show normal nasal NO production rate and the ciliary abnormalities detected by HSVMA are subtle. Elucidating these PCD genotypes highlights potential cases which risk being missed by standard functional tests.

Isolated nexin link defects: Genetic defects encoding the so-called nexin link dynein regulator complex proteins (N-DRCs) result in abnormalities of the nexin link structure connecting the outer doublets. So far, mutations in the genes *CCDC164* [93], *CCDC65* [89], and *GAS8* [45] have been reported. TEM shows no discernible abnormalities. Occasionally, outer doublets are not well aligned in the periphery of the cross section. However, those abnormalities can be also observed in normal individuals. The ciliary beat pattern of patients with isolated nexin link defects is occasionally stiff, but can also appear normal. IF identifying N-DRC defects using antibodies targeting N-DRC proteins such as GAS8 are widely used.

Reduced generation of multiple motile cilia: These individuals show a reduced capacity to generate multiple motile cilia. In patients with mutations in *MCIDAS* [101]respiratory cells usually lack any motile cilia or show only very few motile cilia. MCIDAS appears to be a major regulator of ciliogenesis in multiciliated cells. Downstream of MCIDAS, CCNO [102] is responsible for the function in the deuterosome dependent amplification of basal bodies and basal body docking. Therefore, mutations in *CCNO* result in a defect with a very low number of cilia covering the airway cells. Residual cilia can still show normal cilia motility. TEM can also demonstrate mislocalised basal bodies in the cytoplasm. Because the genetic defects solely alter ciliogenesis in multiciliated cells, the function of nodal monocilia is not altered.

Motile ciliopathies with subtle or no respiratory disease: Recently, genetic defects have been recognized in individuals with motile ciliopathies that result in randomization of left/right body asymmetry and/or male infertility but to date seem to not suffer from the severe chronic destructive airway disease usually reported in PCD. *MNS1* [90], *DNAH9* [10,70] *CCDC11* [103] and *ENCUR* [104] [98] belong to this group.

Genotype-phenotype relationships

Delineation of genotype-phenotype relationships have recently been described in the PCD population. Patients with absent IDA with microtubular disorganisation who have a *CCDC39* or

CCDC40 mutation, have lower body mass indices and worse lung function that declines more rapidly compared to the PCD population with ODA defects and those whom have a *DNAH5* mutation, respectively[9]. Interestingly, patients with microtubular disorganization with IDA defects, many with the *CCDC39* or *CCDC40* mutation, are diagnosed earlier with PCD and even as infants have more airflow limitation compared to those with ODA defects[8]. Patients with the *CCNO* and *MCIDAS* mutations have also been reported to have a worse phenotype with significant bronchiectasis, as well as increased incidence of hydrocephalus[105]. It seems reasonable to recommend increased surveillance and more aggressive treatment plans for these groups. Conversely those with *RSPH1* mutations appear to have less lung function impairment, a later onset of wet cough, a lower prevalence of neonatal respiratory distress and nasal nitric oxide values often outside the diagnostic range[11]. Furthermore, recent data has suggested that mutations in *DNAH9* are associated with milder respiratory phenotypes[6,10] With the increase in discovery of PCD-causing genes, more genotype-phenotype relationships will be elucidated. However, caution is needed when interpreting genotype-phenotype data based on small numbers of patients with variations in any one gene. Moreover, we are aware from diseases such as CF that the phenotype can vary depending on the type of mutation within a gene, and how it effects the protein product. Similar variability is likely within PCD genes.

By definition, patients with PCD have dyskinetic cilia, and our clinical understanding has expected airway cilia to be impacted, as confirmed by HSVMA. Recently described mutations in *ENKUR* and *CCDC11* impact left-right patterning but do not appear to cause pulmonary disease[103,104]. These findings are supported by normal ciliary motility seen by HSVMA and normal TEM in patients with *ENKUR* mutations. Given this, some have questioned whether these patients should be included within the syndrome of PCD due to the significantly different phenotype that does not benefit from the usual PCD management. Conversely, patients with mutations in *CCNO* and *MCIDAS* have reduced generation of motile cilia, rather than dyskinesia [101,102]. These patients have a severe respiratory phenotype and a higher risk of hydrocephalus. However, despite not having dyskinetic cilia, they are best managed in specialist PCD clinics.

Syndromes associated with PCD

Advances in imaging and genotyping have revealed PCD like features in some patients with non-motile ciliopathies. Non-motile primary cilia have a role in cell signalling and sensing of the extracellular environment. Symptoms among ciliopathies include polycystic kidneys, skeletal abnormalities, developmental delay and retinal degeneration. Increasingly abnormalities in motile cilia are described in several non-motile ciliopathies including Jeune syndrome [106], nephronophthisis type 2 (*NPHP2)* [107], Leber Congenital Amaurosis (LCA)[108] and Bardet Biedl syndrome[109].

Some males with X-linked mutations in *RPGR*, which can cause retinitis pigmentosa are also reported to have PCD symptoms[110] associated with dyskinetic ciliary beating[111]. *OFD1* is an X-linked gene associated with several overlapping ciliopathies including oral-facial-digital syndrome and Joubert syndrome. When there is also a motile cilia defect the condition is termed Simpson–Golabi–Behmel syndrome. These individuals suffer from PCD symptoms, overgrowth and can also have abnormally large kidneys, liver and spleen[112].

Management

Since there is no cure for PCD, the aim of treatment is to delay the decline in pulmonary airways disease, whilst maintaining patients' health, social and psychological wellbeing. The variability in clinical manifestations and the scarcity of data from clinical trials make it difficult to formulate a standardised management plan that is suitable for all patients. Management is often extrapolated from other diseases such as CF and chronic rhinosinusitis. Although both CF and PCD inevitably lead to bronchiectasis, their underlying patho-mechanisms and clinical course are different[113 2,114,115] . Response to treatment is likely to vary in PCD and international collaborations are urgently needed to ensure a sufficient number of eligible patients for well-designed treatment trials.

General physicians usually have a poor knowledge of managing rare diseases. Guidelines therefore recommend that patients should be seen several times a year in PCD centres by a specialist multidisciplinary team[22,23,116].

General Health

There is growing evidence for the importance of growth and nutrition in children with PCD. The International PCD Cohort (iPCD) have reported impaired height from an early age, and a longitudinal study has shown reduced growth throughout childhood resulting in loss of body height by adulthood[117,118]. Several studies have observed a relationship between lower nutritional status and worse lung function[2,117,119], although other studies have found no association[115,120]. Furthermore PCD patients have low levels of vitamin D, a finding associated with disease severity in bronchiectasis[119,121]. Knowledge of genotype might indicate the patients at increased risk; e.g. patients with mutations in *CCDC39* and *CCDC40* have been observed to have diminished growth parameters in comparison to *DNAH5*[9].

Patients with PCD often have poor aerobic fitness and regular exercise should be encouraged to improve general wellbeing and perhaps assist in mucus clearance[122,123].

Pulmonary Management

There is recent evidence that patients with PCD have more impaired lung function than those with cystic fibrosis (CF) from early childhood[2]. With the paucity of evidence, treatment is based on more common diseases, in particular CF[116]. Guidelines recommend monitoring to include culture of sputum or cough swabs every 3 to 6 months[22,23,116]. Spirometry is routinely used, but is not as sensitive as lung clearance index (LCI) measured by multiple-breath washout for detecting disease[36,124-127]. LCI is easier to measure than spirometry in young children, but information regarding the relationship between FEV1 and LCI in PCD is conflicting[124-126]. Multiple-breath washout equipment is not always available and testing can be time consuming. LCI is not able to distinguish between individuals with reversible and irreversible structural damage[36]; therefore lung imaging is needed to characterise structural changes. Chest X-rays are probably the most commonly used imaging in clinical practice for assessing structural lung changes in PCD[22,23]. Most infants with PCD have unexpected neonatal respiratory distress despite term birth. The majority of these infants have evidence of lobar collapse, usually involving the upper lobes[25]. However, chest x-rays are not sensitive as a monitoring tool to detect early pulmonary changes. Due to concern over the radiation exposure HRCT is not recommended for routine annual monitoring, but can be used intermittently or when clinically indicated. Typical changes seen in PCD include bronchiectasis, peribronchial thickening, mucus plugging, atelectasis and consolidation or collapse. Lung disease, in particular bronchiectasis, predominantly occurs in the middle and lower lobes, with relative sparing of the upper lobes[115,128-129]. As expected, structural changes increase with age and are associated with lung function[128,129]. There are no scoring systems specific for PCD. Studies have therefore used the scoring systems derived for CF lung disease, (i.e. Brody and Bhalla) [130,131]. However, the structural lung disease in CF and PCD differ, and PCD-specific scoring systems are needed to fully capture changes [132] [114].

To avoid the radiation doses associated with HRCT imaging, there is a growing interest in magnetic resonance imaging (MRI) to assess structural and functional changes[37,133,134]. Further developments of lung MRI may allow longitudinal assessments over time in PCD as well as provide the ability to quantify structural changes on a more regular basis and serve as endpoints in short term intervention therapy trials.Whilst there is virtually no evidence, experts agree that a cornerstone of treatment is airways clearance with the aim of preventing infections, atelectasis, bronchiectasis and respiratory failure [22,23,116]. Techniques include a combination of breathing manoeuvres, positioning, manual percussion, with adjuncts including 'vest therapy' (high frequency chest wall oscillation), PEP devices and oscillatory-PEP. Current practice individualises the method based on disease severity, patient age and preference[135], with clearance usually recommended at least twice daily[22,23,116]. If patients have difficulty clearing secretions, nebulised treatments might augment mucus clearance,

although evidence is poor in PCD. The only clinical trial using hypertonic saline failed to show a significant effect in comparison to isotonic saline; the study was probably underpowered and the outcome measure might not have been ideal, demonstrating the need for multinational trials using sensitive outcome measures[136]. Whilst a small number of case studies have suggested a benefit when using recombinant DNase in patients with PCD[137-139], a large trial of patients with non-CF bronchiectasis reported more frequent pulmonary exacerbations and an increased decline in FEV_1[140,141]. Therefore use of rhDNase in PCD should only be considered on a case-by-case basis.

Prevention and treatment of pulmonary exacerbations is the second facet of pulmonary care, and again evidence is extrapolated from CF[142]. Prevention includes immunization (influenza, pneumococcus etc.), segregation and surveillance programmes. Common isolates include *Haemophilus influenza, Morexella catarrhalis, Steptococcus pneumoniae* and *Pseudomonas aeruginosa*[8,29,30,120,143]. Although treatment of symptomatic infection is intuitive, the treatment of asymptomatic infection is more contentious. In the absence of disease-specific evidence, it is generally accepted that treatment should follow CF guidelines, with modification from the high doses used in CF. Given the toxic effects of some of these antibiotics, adverse events should be monitored in PCD patients. In the absence of strong evidence, a sensible approach is to treat first isolates of pathogens with at least two weeks of antibiotics in an attempt to eradicate. For *P aeruginosa* inhaled antibiotics are usually prescribed; some clinicians will also prescribe an oral antibiotic. Patients who do not respond to treatment may require intravenous therapy. Whilst prophylactic use of azithromycin can be beneficial in CF and non-CF bronchiectasis, the evidence for use in PCD is awaiting results from a recent trial[144].

The role of thoracic surgery in PCD is unclear, but in the authors experience is rarely indicated. Lung transplantation is an option in patients with end stage disease, and has been performed in a number of patients in the US[28]. Recent studies indicate that transplantation is undertaken exceptionally rarely in Eurpope[26,145]. Transplantation requires careful planning particularly in patients with situs abnormalities.

Upper airways and ears

Since the majority of patients with PCD have chronic rhino-sinusitis, otitis media with effusion, and chronic otitis media[1,26,27], an otolaryngologist should be part of the PCD multidisciplinary team.

Perennial nasal discharge often starts from early infancy, and can be debilitating, affecting quality of life[4,5,146]. Anecdotally, sino-nasal rinsing with saline can relieve symptoms. Topical nasal steroids are often used although there is no evidence of efficacy[147].

Management of the ears aims to improve hearing and prevent irreversible damage of the tympanic membranes. Ear disease is very common, particularly in young children. Symptoms often improve with age, although some adults continue to require hearing aids[27,148,149]. The conductive hearing loss associated with middle ear effusions can delay speech acquisition, impair learning, and create social isolation. Hearing should therefore be monitored regularly using age-appropriate methods. Oral antibiotic treatment of acute otitis media may prevent complications, and topical antibiotics can be prescribed for ear discharge. In PCD, the use of ventilation tubes is controversial because troublesome mucopurulent discharge is reportedly more common than in the general population (30% versus 5%), however, randomised studies have not been undertaken[149,150]. The ERS guidelines recommend hearing aids rather than ventilation tubes, until such time as the hearing loss resolves[22]. Patients with mutations associated with central complex defects (Table 1), have been reported in one study to have more ear-related problems. This group might benefit from more regular monitoring[149].

Cardiac disease

Echocardiogram and abdominal ultrasounds should follow diagnosis in patients with genes known to effect laterality (Table 1).

Fertility

Infertility is common in PCD and patients need access to specialist care for assisted fertility[7]. There is increasing data to guide which patients require counselling regarding fertility, for example, patients with mutations in *CCDC114* appear to have normal fertility[14], whilst limited data suggests male infertility is highly likely in patients with mutations in *CCDC39, CCDC40, DNAAF1* and *LRRC6*[7]. A number of men have successfully fathered children following intracytoplasmic sperm injection (ICSI). Information regarding pregnancy is limited, but anecdotally, a number of women with PCD have successfully delivered healthy babies. There is dogma that women are at high risk of ectopic pregnancy, but experience of the authors as well as lack of epidemiological evidence cast doubt on this[7].

Potential novel therapies

There is much interest in genetic correction of mutated PCD genes as a potential cure. Several studies have reported partial recovery of ciliary beating investigated using lentiviral transfection or TALENs (transcription activator-like effector endonucleases) in ciliated cell culture models and transgenic mouse models[151-153]. Other gene editing techniques exist, for example read through therapies[154] and CRISPR-Cas9. With recent successes in cystic fibrosis, and the increased

understanding of the genetic basis of PCD, there are a number of researchers investigating targeted small molecule therapies.

Summary

In summary, PCD is a syndrome caused by genetic mutations effecting motile cilia, causing disease of the upper and lower airways. Next generation sequencing has led to advances in the discovery of new PCD genes over the past decade, and over 40 genes are now reported to cause this rare disease. This remarkable progress is impacting diagnostic capabilities, genetic counselling and has the potential to lead to new therapeutic options and personalised care.

There is increasing understanding of the influence of genotype on clinical presentation, although research is limited by the number of patients with mutations in any one gene. There is no gold standard diagnostic test, and a combination of nNO screening, analysis of cilia ultrastructure by EM; analysis of ciliary function by HSVM, Immuno-labelling of ciliary proteins (IF) and genetic testing can be used. Because there are a large number of implicated genes and mutations, and because many genes have yet to be found, genetic testing is not yet a definitive diagnostic test for PCD. There is no cure for PCD and management focuses on careful monitoring and physiotherapy as well as treatment of infections. The lack of a definitive diagnostic test and absence of specific treatments highlight the importance of collaborative international research for this rare disease.

Discussions between patient representatives and clinicians are needed to clarify the definition of PCD in this genomic age. Whilst previously we presumed that all patients would have pulmonary involvement, there have been recent discoveries of genes (e.g.ENKUR) affecting motile cilia that cause situs inversus in the absence of significant chest disease. Should these patients be included in the syndrome? Further study is also indicated in patients with mutations in genes where there is ciliary dyskinesia, but some mucociliary clearance is maintained (e.g. DNAH9). Conversely patients with mutations in CCNO and MCIDAS, do not have dyskinetic cilia. Rather, their relatively severe respiratory phenotype, caused by reduced numbers of motile cilia, is typical of PCD. Should this group of patients have a different label for their disease, or is it clinically sensible for them to be identified as having PCD? There is large heterogeneity and we are at the tip of the iceberg in understanding the disease.

Table1. Genetic defects in PCD, and the associated phenotypes where these differ from 'classical' PCD

Functional defect	Gene	OMIM	Predominant ultrastructural defect by TEM	Predominant HSVMA	Phenotype notes	Ref.
Randomization of L/R asymmetry						
ODA	*DNAH5*	603335	ODA	Static		64
	*DNAH11**	603339	Normal[$]	Hyperfrequent/ stiff at base		65
	DNAI1	604366	ODA	Static		66
	DNAI2	605483	ODA	Static		67
	DNAL1	610062	ODA	Static		68
	NME8	607421	ODA	Static		69
	DNAH9	603330	ODA-type 1	Normal/ stiff at top	Normal NO/ milder resp. phenotype	10,70
ODA Docking	*CCDC114*	615038	ODA	Static	Preserved fertility	14,72
	ARMC4	615408	ODA	Static		73
	CCDC151	615956	ODA	Static		75
	CCDC103	614677	ODA/ normal[$$]	Static/ normal[2]	Normal NO/ milder resp. phenotype[$$]	77
	TTC25	617095	ODA	Static		76
	MNS1	610766	ODA	Normal	Milder resp. phenotype	90
Pre-assembly Factor	*DNAAF1*	613190	ODA + IDA	Static		79,80
	DNAAF2	612517	ODA + IDA	Static		81
	DNAAF3	614566	ODA + IDA	Static		82
	DNAAF4	608706	ODA + IDA	Static		83
	DNAAF5	614864	ODA + IDA	Static		84
	LRRC6	614930	ODA + IDA	Static		85
	ZMYND10	607070	ODA + IDA	Static		86,87
	SPAG1	603395	ODA + IDA	Static		88
	C21ORF59	615494	ODA + IDA	Static		89
	PIH1D3	300933	ODA + IDA	Static		59,60
	CFAP300	618058	ODA + IDA	Static		155
Axonemal ruler	*CCDC39*	613798	MTD + IDA	Reduced bend and amplitude	More severe resp. pheno and poorer nutrition[8,9]	91
	CCDC40	613798	MTD + IDA	Reduced bend and amplitude	More severe resp. pheno and poorer nutrition[8,9]	92,156
N-DRC	*CCDC164*	615288	MTD /normal	Hyperfrequent/ dyskinetic/ normal	Situs solitus	93
	CCDC65	611088	MTD /normal	Hyperfrequent/ dyskinetic/ normal	Situs solitus	89,157
	GAS8	605178	MTD /normal	Hyperfrequent/ dyskinetic/ normal	Situs solitus	40,88 45,94
Radial spoke Subunit	*RSPH1*	609314	CC/ normal	Circling[$$$]	Normal NO + milder resp. phenotype. Situs solitus	95
	RSPH4A	612647	CC/ normal	Circling[$$$]	Situs solitus, increased chance of OM[132149]	158
	RSPH9	612648	CC/ normal	Circling[$$$]	Situs solitus; increased chance of OM[149]	96
	RSPH3	615876	CC/ normal	Circling[$$$]	Situs solitus; increased chance of OM[149]	97
	DNAJB13	610263	CC/ normal	Circling[$$$]		98

Central pair	*HYDIN*	610812	C2b projection/ normal	Dyskinetic[$$$]	Situs solitus	*100*
Subunit	*STK36*	607652	normal	Dyskinetic[$$$]		46
Other	*CCNO*	607752	Mislocalisation of basal body + reduction of cilia	RGMC	More severe + Hydrocepahlus	102
	*MCIDAS**	614086	Mislocalisation of basal body + reduction of cilia ODA+IDA	RGMC	More severe + Hydrocepahlus	101
	CCDC11	614759	normal	normal	Normal/mild?	103
	ENKUR	611025	N.A.	normal	Normal/mild?	104
	GAS2L2	611398	normal	Normal/ disorientation		44
	*LRRC56**	618227	normal	Dyskinetic		78

TEM: transmission electron microscopy; HSVMA: high-speed video microscopy analysis; RGMC: reduced generation of multiple motile cilia; ODA: outer dynein arm; IDA: Inner dynein arm; N-DRC: nexin-dynein regulatory complex NO: nasal nitric oxide; MTD: Microtubular disorganization; N.A.: not applicable; OM: otitis media

* Not always detectable by immunofluorescence using targeted antibodies

[$] Ultrastructural defects can be identified by electron tomography

[$$]Refers only to the His154Pro missense mutation

[$$$]When viewed from above. Sometimes can appear dyskinetic or more normal

Figure legends

Figure 1: Over 40 genes have been reported to cause PCD. This figure summarises the genes which can be associated with PCD by effecting ciliary proteins, transport of those proteins, or docking of structures. ODA= outer dynein arm; CP= central pair; N-DCR=nexin-dynein regulatory complex.

Figure 2: Transmission electron microscopy images of: A) Outer dynein arm defects B) Inner and outer dynein arm defects C) Microtubular disorganisation and inner dynein arm defect D) Normal ultrastructure E) Central complex defect showing separation of the central pair and translocation of an outer microtubular doublet.

References

1. Goutaki M, Meier AB, Halbeisen FS, et al. Clinical manifestations in primary ciliary dyskinesia: systematic review and meta-analysis. *Eur Respir J,* 2016; **48**(4): 1081-95.
2. Halbeisen FS, Goutaki M, Spycher BD, et al. Lung function in patients with primary ciliary dyskinesia: an iPCD Cohort study. *Eur Respir J* 2018; **52**(2).
3. Marthin JK, Petersen N, Skovgaard LT, Nielsen KG. Lung function in patients with primary ciliary dyskinesia: a cross-sectional and 3-decade longitudinal study. *Am J Respir Crit Care Med* 2010; **181**(11): 1262-8.
4. Lucas JS, Behan L, Dunn Galvin A, et al. A quality-of-life measure for adults with primary ciliary dyskinesia: QOL-PCD. *Eur Respir J* 2015; **46**(2): 375-83.
5. Behan L, Leigh MW, Dell SD, Dunn Galvin A, Quittner AL, Lucas JS. Validation of a health-related quality of life instrument for primary ciliary dyskinesia (QOL-PCD). *Thorax* 2017; Sep;72(9):832-839.
6. Best S, Shoemark A, Rubbo B, et al. Risk factors for situs defects and congenital heart disease in primary ciliary dyskinesia. *Thorax* 2019 Feb;74(2):203-205
7. Vanaken GJ, Bassinet L, Boon M, et al. Infertility in an adult cohort with primary ciliary dyskinesia: phenotype-gene association. *Eur Respir J* 2017; **50**(5).
8. Davis SD, Ferkol TW, Rosenfeld M, et al. Clinical features of childhood primary ciliary dyskinesia by genotype and ultrastructural phenotype. *Am J Respir Crit Care Med* 2015; **191**(3): 316-24.
9. Davis SD, Rosenfeld M, Lee HS, et al. Primary Ciliary Dyskinesia: Longitudinal Study of Lung Disease by Ultrastructure Defect and Genotype. *Am J Respir Crit Care Med* 2019; **199**(2): 190-8.
10. Fassad MR, Shoemark A, Legendre M, et al. Mutations in Outer Dynein Arm Heavy Chain DNAH9 Cause Motile Cilia Defects and Situs Inversus. *Am J Hum Genet* 2018; **103**(6): 995-1008.
11. Knowles MR, Ostrowski LE, Leigh MW, et al. Mutations in RSPH1 cause primary ciliary dyskinesia with a unique clinical and ciliary phenotype. *Am J Respir Crit Care Med* 2014; **189**(6): 707-17.
12. Kuehni CE, Frischer T, Strippoli MP, et al. Factors influencing age at diagnosis of primary ciliary dyskinesia in European children. *Eur Respir J* 2010; **36**(6): 1248-58.
13. O'Callaghan C, Chetcuti P, Moya E. High prevalence of primary ciliary dyskinesia in a British Asian population. *Arch Dis Child* 2010; **95**(1): 51-2.
14. Onoufriadis A, Paff T, Antony D, et al. Splice-site mutations in the axonemal outer dynein arm docking complex gene CCDC114 cause primary ciliary dyskinesia. *Am J Hum Genet* 2013; **92**(1): 88-98.
15. Ferkol TW, Puffenberger EG, Lie H, et al. Primary ciliary dyskinesia-causing mutations in Amish and Mennonite communities. *J Pediatr* 2013; **163**(2): 383-7.
16. Casey JP, McGettigan PA, Healy F, et al. Unexpected genetic heterogeneity for primary ciliary dyskinesia in the Irish Traveller population. *Eur J Hum Genetics* 2015; **23**(2): 210-7.
17. Hammoudeh S, Gadelhak W, Janahi IA. Primary ciliary dyskinesia among Arabs: Where do we go from here? *Paed Respir Rev* 2019; **29**: 19-22.
18. Rumman N, Jackson C, Collins S, Goggin P, Coles J, Lucas JS. Diagnosis of primary ciliary dyskinesia: potential options for resource-limited countries. *Eur Respir Rev* 2017; **26**(143).
19. Behan L, Dunn Galvin A, Rubbo B, et al. Diagnosing primary ciliary dyskinesia: an international patient perspective. *Eur Respir J* 2016; **48**(4): 1096-107.
20. Behan L, Dimitrov BD, Kuehni CE, et al. PICADAR: a diagnostic predictive tool for primary ciliary dyskinesia. *Eur Respir J* 2016; **47**(4): 1103-12.
21. Leigh MW, Ferkol TW, Davis SD, et al. Clinical Features and Associated Likelihood of Primary Ciliary Dyskinesia in Children and Adolescents. *Ann Am Thorac Soc* 2016; **13**(8): 1305-13.

22. Barbato A, Frischer T, Kuehni CE, et al. Primary ciliary dyskinesia: a consensus statement on diagnostic and treatment approaches in children. *Eur Respir J* 2009; **34**(6): 1264-76.
23. Shapiro AJ, Zariwala MA, Ferkol T, et al. Diagnosis, monitoring, and treatment of primary ciliary dyskinesia: PCD foundation consensus recommendations based on state of the art review. *Pediatr Pulmonol* 2016; **51**(2): 115-32.
24. Knowles MR, Daniels LA, Davis SD, Zariwala MA, Leigh MW. Primary ciliary dyskinesia. Recent advances in diagnostics, genetics, and characterization of clinical disease. *Am J Respir Crit Care Med* 2013; **188**(8): 913-22.
25. Mullowney T, Manson D, Kim R, Stephens D, Shah V, Dell S. Primary ciliary dyskinesia and neonatal respiratory distress. *Pediatrics* 2014; **134**(6): 1160-6.
26. Frija-Masson J, Bassinet L, Honore I, et al. Clinical characteristics, functional respiratory decline and follow-up in adult patients with primary ciliary dyskinesia. *Thorax* 2017; **72**(2): 154-60.
27. Sommer JU, Schafer K, Omran H, et al. ENT manifestations in patients with primary ciliary dyskinesia: prevalence and significance of otorhinolaryngologic co-morbidities. *Eur Arch Otorhinolaryngol* 2011; **268**(3): 383-8.
28. Noone PG, Leigh MW, Sannuti A, et al. Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 2004; **169**(4): 459-67.
29. Alanin MC, Nielsen KG, von Buchwald C, et al. A longitudinal study of lung bacterial pathogens in patients with primary ciliary dyskinesia. *Clin. Microbiol. Infect* 2015; **21**(12): 1093.e1-7.
30. Rogers GB, Carroll MP, Zain NM, et al. Complexity, temporal stability, and clinical correlates of airway bacterial community composition in primary ciliary dyskinesia. *J Clinl Micro* 2013; **51**(12): 4029-35.
31. Brown DE, Pittman JE, Leigh MW, Fordham L, Davis SD. Early lung disease in young children with primary ciliary dyskinesia. *Ped Pulm* 2008; **43**(5): 514-6.
32. Kennedy MP, Omran H, Leigh MW, et al. Congenital heart disease and other heterotaxic defects in a large cohort of patients with primary ciliary dyskinesia. *Circulation* 2007; **115**(22): 2814-21.
33. Shapiro AJ, Davis SD, Ferkol T, et al. Laterality defects other than situs inversus totalis in primary ciliary dyskinesia: insights into situs ambiguus and heterotaxy. *Chest* 2014; **146**(5): 1176-86.
34. Lucas JS, Barbato A, Collins SA, et al. European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia. *Eur Respir J* 2017; **49**(1).
35. Rademacher J, Buck A, Schwerk N, et al. Nasal Nitric Oxide Measurement and a Modified PICADAR Score for the Screening of Primary Ciliary Dyskinesia in Adults with Bronchiectasis. *Pneumologie* 2017; **71**(8): 543-8.
36. Boon M, Vermeulen FL, Gysemans W, Proesmans M, Jorissen M, De Boeck K. Lung structure-function correlation in patients with primary ciliary dyskinesia. *Thorax* 2015; **70**(4): 339-45.
37. Nyilas S, Bauman G, Pusterla O, et al. Structural and Functional Lung Impairment in Primary Ciliary Dyskinesia. Assessment with Magnetic Resonance Imaging and Multiple Breath Washout in Comparison to Spirometry. *Ann Am Thorac Soc* 2018; **15**(12): 1434-42.
38. Shapiro AJ, Davis SD, Polineni D, et al. Diagnosis of Primary Ciliary Dyskinesia. An Official American Thoracic Society Clinical Practice Guideline. *Am J Respir Crit Care Med* 2018; **197**(12): e24-e39.
39. Walker WT, Jackson CL, Lackie PM, Hogg C, Lucas JS. Nitric oxide in primary ciliary dyskinesia. *Eur Respir J* 2012; **40**(4): 1024-32.
40. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005; **171**(8): 912-30.
41. Wodehouse T, Kharitonov SA, Mackay IS, Barnes PJ, Wilson R, Cole PJ. Nasal nitric oxide measurements for the screening of primary ciliary dyskinesia. *Eur Respir J* 2003; **21**(1): 43-7.
42. Marthin JK, Nielsen KG. Choice of nasal nitric oxide technique as first-line test for primary ciliary dyskinesia. *Eur Respir J* 2011; **37**(3): 559-65.

43. Shoemark A, Moya E, Hirst RA, et al. High prevalence of CCDC103 p.His154Pro mutation causing primary ciliary dyskinesia disrupts protein oligomerisation and is associated with normal diagnostic investigations. *Thorax* 2018; **73**(2): 157-66.
44. Bustamante-Marin XM, Yin WN, Sears PR, et al. Lack of GAS2L2 Causes PCD by Impairing Cilia Orientation and Mucociliary Clearance. *American journal of human genetics* 2019; **104**(2): 229-45.
45. Olbrich H, Cremers C, Loges NT, et al. Loss-of-Function GAS8 Mutations Cause Primary Ciliary Dyskinesia and Disrupt the Nexin-Dynein Regulatory Complex. *Am J Hum Genet* 2015; **97**(4): 546-54.
46. Edelbusch C, Cindric S, Dougherty GW, et al. Mutation of serine/threonine protein kinase 36 (STK36) causes primary ciliary dyskinesia with a central pair defect. *Hum Mutat.* 2017;**38**(8):964-96.
47. Harris A, Bhullar E, Gove K, et al. Validation of a portable nitric oxide analyzer for screening in primary ciliary dyskinesias. *BMC Pulmon Med* 2014; **14**: 18.
48. Chilvers MA, Rutman A, O'Callaghan C. Ciliary beat pattern is associated with specific ultrastructural defects in primary ciliary dyskinesia. *J Allergy Clin Immunol* 2003; **112**(3): 518-24.
49. Raidt J, Wallmeier J, Hjeij R, et al. Ciliary beat pattern and frequency in genetic variants of primary ciliary dyskinesia. *Eur Respir J* 2014; **44**(6): 1579-88.
50. Jackson CL, Behan L, Collins SA, et al. Accuracy of diagnostic testing in primary ciliary dyskinesia. *Eur Respir J* 2016; **47**(3): 837-48.
51. Fliegauf M, Olbrich H, Horvath J, et al. Mislocalization of DNAH5 and DNAH9 in respiratory cells from patients with primary ciliary dyskinesia. *American journal of respiratory and critical care medicine* 2005; **171**(12): 1343-9.
52. Shoemark A, Frost E, Dixon M, et al. Accuracy of Immunofluorescence in the Diagnosis of Primary Ciliary Dyskinesia. *Am J Respir Crit Care Med* 2017; **196**(1): 94-101.
53. Afzelius BA. A human syndrome caused by immotile cilia. *Science* 1976; **193**(4250): 317-9.
54. Boon M, Smits A, Cuppens H, et al. Primary ciliary dyskinesia: critical evaluation of clinical symptoms and diagnosis in patients with normal and abnormal ultrastructure. *Orphanet J Rare Dis.* 2014; **9:**11.
55. Knowles MR, Leigh MW, Carson JL, et al. Mutations of DNAH11 in patients with primary ciliary dyskinesia with normal ciliary ultrastructure. *Thorax* 2012; **67**(5): 433-41.
56. Marthin JK, Mortensen J, Pressler T, Nielsen KG. Pulmonary radioaerosol mucociliary clearance in diagnosis of primary ciliary dyskinesia. *Chest* 2007; **132**(3): 966-76.
57. Walker WT, Young A, Bennett M, et al. Pulmonary radioaerosol mucociliary clearance in primary ciliary dyskinesia. *Eur Respir J* 2014; **44**(2): 533-5.
58. Shoemark A, Burgoyne T, Kwan R, et al. Primary ciliary dyskinesia with normal ultrastructure: three-dimensional tomography detects absence of DNAH11. *Eur Respir J* 2018; **51**(2).
59. Paff T, Loges NT, Aprea I, et al. Mutations in PIH1D3 Cause X-Linked Primary Ciliary Dyskinesia with Outer and Inner Dynein Arm Defects. *Am J Hum Genet* 2017; **100**(1): 160-8.
60. Olcese C, Patel MP, Shoemark A, et al. X-linked primary ciliary dyskinesia due to mutations in the cytoplasmic axonemal dynein assembly factor PIH1D3. *Nat Commun.* 2017; **8:**14279.
61. Bush A, Payne D, Pike S, Jenkins G, Henke MO, Rubin BK. Mucus properties in children with primary ciliary dyskinesia: comparison with cystic fibrosis. *Chest* 2006; **129**(1): 118-23.
62. Ratjen F, Waters V, Klingel M, et al. Changes in airway inflammation during pulmonary exacerbations in patients with cystic fibrosis and primary ciliary dyskinesia. *Eur Respir J* 2016; **47**(3): 829-36.
63. Cockx M, Gouwy M, Ruytinx P, et al. Monocytes from patients with Primary Ciliary Dyskinesia show enhanced inflammatory properties and produce higher levels of pro-inflammatory cytokines. *Scientific Reports* 2017; **7**(1): 14657.
64. Olbrich H, Haffner K, Kispert A, et al. Mutations in DNAH5 cause primary ciliary dyskinesia and randomization of left-right asymmetry. *Nat Genetics* 2002; **30**(2): 143-4.
65. Bartoloni L, Blouin JL, Pan Y, et al. Mutations in the DNAH11 (axonemal heavy chain dynein type 11) gene cause one form of situs inversus totalis and most likely primary ciliary dyskinesia. *Proc Natl Acad Sci U S A.* 2002; **99**(16): 10282-6.

66. Pennarun G, Escudier E, Chapelin C, et al. Loss-of-function mutations in a human gene related to Chlamydomonas reinhardtii dynein IC78 result in primary ciliary dyskinesia. *Am J Hum Genet* 1999; **65**(6): 1508-19.
67. Loges NT, Olbrich H, Fenske L, et al. DNAI2 mutations cause primary ciliary dyskinesia with defects in the outer dynein arm. *Am J Hum Genet* 2008; **83**(5): 547-58.
68. Mazor M, Alkrinawi S, Chalifa-Caspi V, et al. Primary ciliary dyskinesia caused by homozygous mutation in DNAL1, encoding dynein light chain 1. *Am J Hum Genet* 2011; **88**(5): 599-607.
69. Duriez B, Duquesnoy P, Escudier E, et al. A common variant in combination with a nonsense mutation in a member of the thioredoxin family causes primary ciliary dyskinesia. *Proc Natl Acad Sci U S A.* 2007; **104**(9): 3336-41.
70. Loges NT, Antony D, Maver A, et al. Recessive DNAH9 Loss-of-Function Mutations Cause Laterality Defects and Subtle Respiratory Ciliary-Beating Defects. *Am J Hum Genet* 2018; **103**(6): 995-1008.
71. Dougherty GW, Loges NT, Klinkenbusch JA, et al. DNAH11 Localization in the Proximal Region of Respiratory Cilia Defines Distinct Outer Dynein Arm Complexes. *Am J Respir Cell Mol Biol* 2016; **55**(2): 213-24.
72. Knowles MR, Leigh MW, Ostrowski LE, et al. Exome sequencing identifies mutations in CCDC114 as a cause of primary ciliary dyskinesia. *Am J Hum Genet* 2013; **92**(1): 99-106.
73. Hjeij R, Lindstrand A, Francis R, et al. ARMC4 mutations cause primary ciliary dyskinesia with randomization of left/right body asymmetry. *Am J Hum Genet* 2013; **93**(2): 357-67.
74. Alsaadi MM, Erzurumluoglu AM, Rodriguez S, et al. Nonsense mutation in coiled-coil domain containing 151 gene (CCDC151) causes primary ciliary dyskinesia. *Hum Mutat* 2014; **35**(12): 1446-8.
75. Hjeij R, Onoufriadis A, Watson CM, et al. CCDC151 mutations cause primary ciliary dyskinesia by disruption of the outer dynein arm docking complex formation. *Am J Hum Genet* 2014; **95**(3): 257-74.
76. Wallmeier J, Shiratori H, Dougherty GW, et al. TTC25 Deficiency Results in Defects of the Outer Dynein Arm Docking Machinery and Primary Ciliary Dyskinesia with Left-Right Body Asymmetry Randomization. *Am J Hum Genet* 2016; **99**(2): 460-9.
77. Panizzi JR, Becker-Heck A, Castleman VH, et al. CCDC103 mutations cause primary ciliary dyskinesia by disrupting assembly of ciliary dynein arms. *Nat Genetics* 2012; **44**(6): 714-9.
78. Bonnefoy S, Watson CM, Kernohan KD, et al. Biallelic Mutations in LRRC56, Encoding a Protein Associated with Intraflagellar Transport, Cause Mucociliary Clearance and Laterality Defects. *Am J Hum Genet* 2018; **103**(5): 727-39.
79. Duquesnoy P, Escudier E, Vincensini L, et al. Loss-of-function mutations in the human ortholog of Chlamydomonas reinhardtii ODA7 disrupt dynein arm assembly and cause primary ciliary dyskinesia. *Am J Hum Genet* 2009; **85**(6): 890-6.
80. Loges NT, Olbrich H, Becker-Heck A, et al. Deletions and point mutations of LRRC50 cause primary ciliary dyskinesia due to dynein arm defects. *Am J Hum Genet* 2009; **85**(6): 883-9.
81. Omran H, Kobayashi D, Olbrich H, et al. Ktu/PF13 is required for cytoplasmic pre-assembly of axonemal dyneins. *Nature* 2008; **456**(7222): 611-6.
82. Mitchison HM, Schmidts M, Loges NT, et al. Mutations in axonemal dynein assembly factor DNAAF3 cause primary ciliary dyskinesia. *Nat Genetics* 2012; **44**(4): 381-9, s1-2.
83. Tarkar A, Loges NT, Slagle CE, et al. DYX1C1 is required for axonemal dynein assembly and ciliary motility. *Nat Genetics* 2013; **45**(9): 995-1003.
84. Horani A, Druley TE, Zariwala MA, et al. Whole-exome capture and sequencing identifies HEATR2 mutation as a cause of primary ciliary dyskinesia. *Am J Hum Genet* 2012; **91**(4): 685-93.
85. Kott E, Duquesnoy P, Copin B, et al. Loss-of-function mutations in LRRC6, a gene essential for proper axonemal assembly of inner and outer dynein arms, cause primary ciliary dyskinesia. *Am J Hum Genet* 2012; **91**(5): 958-64.

86. Moore DJ, Onoufriadis A, Shoemark A, et al. Mutations in ZMYND10, a gene essential for proper axonemal assembly of inner and outer dynein arms in humans and flies, cause primary ciliary dyskinesia. *Am J Hum Genet* 2013; **93**(2): 346-56.
87. Zariwala MA, Gee HY, Kurkowiak M, et al. ZMYND10 is mutated in primary ciliary dyskinesia and interacts with LRRC6. *Am J Hum Genet* 2013; **93**(2): 336-45.
88. Knowles MR, Ostrowski LE, Loges NT, et al. Mutations in SPAG1 cause primary ciliary dyskinesia associated with defective outer and inner dynein arms. *Am J Hum Genet* 2013; **93**(4): 711-20.
89. Austin-Tse C, Halbritter J, Zariwala MA, et al. Zebrafish Ciliopathy Screen Plus Human Mutational Analysis Identifies C21orf59 and CCDC65 Defects as Causing Primary Ciliary Dyskinesia. *Am J Hum Genet* 2013; **93**(4): 672-86.
90. Ta-Shma A, Hjeij R, Perles Z, et al. Homozygous loss-of-function mutations in MNS1 cause laterality defects and likely male infertility. *PLoS Genetics* 2018; **14**(8): e1007602.
91. Merveille AC, Davis EE, Becker-Heck A, et al. CCDC39 is required for assembly of inner dynein arms and the dynein regulatory complex and for normal ciliary motility in humans and dogs. *Nat Genetics* 2011; **43**(1): 72-8.
92. Becker-Heck A, Zohn IE, Okabe N, et al. The coiled-coil domain containing protein CCDC40 is essential for motile cilia function and left-right axis formation. *Nat Genetics* 2011; **43**(1): 79-84.
93. Wirschell M, Olbrich H, Werner C, et al. The nexin-dynein regulatory complex subunit DRC1 is essential for motile cilia function in algae and humans. *Nat Genetics* 2013; **45**(3): 262-8.
94. Jeanson L, Thomas L, Copin B, et al. Mutations in GAS8, a Gene Encoding a Nexin-Dynein Regulatory Complex Subunit, Cause Primary Ciliary Dyskinesia with Axonemal Disorganization. *Hum Mutat* 2016; **37**(8): 776-85.
95. Kott E, Legendre M, Copin B, et al. Loss-of-function mutations in RSPH1 cause primary ciliary dyskinesia with central-complex and radial-spoke defects. *Am J Hum Genet* 2013; **93**(3): 561-70.
96. Castleman VH, Romio L, Chodhari R, et al. Mutations in radial spoke head protein genes RSPH9 and RSPH4A cause primary ciliary dyskinesia with central-microtubular-pair abnormalities. *Am J Hum Genet* 2009; **84**(2): 197-209.
97. Jeanson L, Copin B, Papon JF, et al. RSPH3 Mutations Cause Primary Ciliary Dyskinesia with Central-Complex Defects and a Near Absence of Radial Spokes. *Am J Hum Genet* 2015; **97**(1): 153-62.
98. El Khouri E, Thomas L, Jeanson L, et al. Mutations in DNAJB13, Encoding an HSP40 Family Member, Cause Primary Ciliary Dyskinesia and Male Infertility. *Am J Hum Genet* 2016; **99**(2): 489-500.
99. Frommer A, Hjeij R, Loges NT, et al. Immunofluorescence Analysis and Diagnosis of Primary Ciliary Dyskinesia with Radial Spoke Defects. *Am J Respir Cell Mol Biol* 2015; **53**(4): 563-73.
100. Olbrich H, Schmidts M, Werner C, et al. Recessive HYDIN mutations cause primary ciliary dyskinesia without randomization of left-right body asymmetry. *Am J Hum Genet* 2012; **91**(4): 672-84.
101. Boon M, Wallmeier J, Ma L, et al. MCIDAS mutations result in a mucociliary clearance disorder with reduced generation of multiple motile cilia. *Nat Commun* 2014; **5**: 4418.
102. Wallmeier J, Al-Mutairi DA, Chen CT, et al. Mutations in CCNO result in congenital mucociliary clearance disorder with reduced generation of multiple motile cilia. *Nat Genetics* 2014; **46**(6): 646-51.
103. Silva E, Betleja E, John E, et al. Ccdc11 is a novel centriolar satellite protein essential for ciliogenesis and establishment of left-right asymmetry. *Mol Biol Cell* 2016; **27**(1): 48-63.
104. Sigg MA, Menchen T, Lee C, et al. Evolutionary Proteomics Uncovers Ancient Associations of Cilia with Signaling Pathways. *Dev Cell* 2017; **43**(6): 744-62.e11.
105. Amirav I, Wallmeier J, Loges NT, et al. Systematic Analysis of CCNO Variants in a Defined Population: Implications for Clinical Phenotype and Differential Diagnosis. *Hum Mutat* 2016; **37**(4): 396-405.

106. Emiralioglu N, Wallmeier J, Olbrich H, Omran H, Ozcelik U. DYNC2H1 mutation causes Jeune syndrome and recurrent lung infections associated with ciliopathy. *Clin Respir J* 2018; **12**(3): 1017-20.
107. Moalem S, Keating S, Shannon P, et al. Broadening the ciliopathy spectrum: motile cilia dyskinesia, and nephronophthisis associated with a previously unreported homozygous mutation in the INVS/NPHP2 gene. *Am J Med Genet Part A 2013*; **161a**(7): 1792-6.
108. Papon JF, Perrault I, Coste A, et al. Abnormal respiratory cilia in non-syndromic Leber congenital amaurosis with CEP290 mutations. *J Med Genet* 2010; **47**(12): 829-34.
109. Shoemark A, Dixon M, Beales PL, Hogg CL. Bardet Biedl syndrome: motile ciliary phenotype. *Chest* 2015; **147**(3): 764-70.
110. Moore A, Escudier E, Roger G, et al. RPGR is mutated in patients with a complex X linked phenotype combining primary ciliary dyskinesia and retinitis pigmentosa. *J Med Genetics* 2006; **43**(4): 326-33.
111. Bukowy-Bieryllo Z, Zietkiewicz E, Loges NT, et al. RPGR mutations might cause reduced orientation of respiratory cilia. *Ped Pulm* 2013; **48**(4): 352-63.
112. Budny B, Chen W, Omran H, et al. A novel X-linked recessive mental retardation syndrome comprising macrocephaly and ciliary dysfunction is allelic to oral-facial-digital type I syndrome. *Hum Genetics* 2006; **120**(2): 171-8.
113. Lucas JS, Carroll M. Primary ciliary dyskinesia and cystic fibrosis: different diseases require different treatment. *Chest* 2014; **145**(4): 674-6.
114. Robinson P, Morgan L. Bronchiectasis in PCD looks different to CF on CT scan. *Multidisc Respir Med* 2018; **13**(Suppl 1): 24.
115. Cohen-Cymberknoh M, Simanovsky N, Hiller N, Hillel AG, Shoseyov D, Kerem E. Differences in disease expression between primary ciliary dyskinesia and cystic fibrosis with and without pancreatic insufficiency. *Chest* 2014; **145**(4): 738-44.
116. Lucas JS, Alanin MC, Collins S, et al. Clinical care of children with primary ciliary dyskinesia. *Expert Rev Respir Med* 2017; **11**(10): 779-90.
117. Goutaki M, Halbeisen FS, Spycher BD, et al. Growth and nutritional status, and their association with lung function: a study from the international Primary Ciliary Dyskinesia Cohort. *Eur Respir J* 2017; **50**(6).
118. Svobodova T, Djakow J, Zemkova D, Cipra A, Pohunek P, Lebl J. Impaired Growth during Childhood in Patients with Primary Ciliary Dyskinesia. *Int J Endocrinol* 2013; **2013**: 731423.
119. Marino LV, Harris A, Johnstone C, et al. Characterising the nutritional status of children with primary ciliary dyskinesia. *Clin Nutr. 2018. pii: S0261-5614(18)32428-2*
120. Maglione M, Bush A, Nielsen KG, et al. Multicenter analysis of body mass index, lung function, and sputum microbiology in primary ciliary dyskinesia. *Ped Pulm* 2014; **49**(12): 1243-50.
121. Mirra V, Caffarelli C, Maglione M, et al. Hypovitaminosis D: a novel finding in primary ciliary dyskinesia. *Ital J Pediatr. 2015;**41:**14*
122. Madsen A, Green K, Buchvald F, Hanel B, Nielsen KG. Aerobic fitness in children and young adults with primary ciliary dyskinesia. *PloS One* 2013; **8**(8): e71409.
123. Valerio G, Giallauria F, Montella S, et al. Cardiopulmonary assessment in primary ciliary dyskinesia. *Eur J Clin Invest* 2012; **42**(6): 617-22.
124. Green K, Buchvald FF, Marthin JK, Hanel B, Gustafsson PM, Nielsen KG. Ventilation inhomogeneity in children with primary ciliary dyskinesia. *Thorax* 2012; **67**(1): 49-53.
125. Irving SJ, Ives A, Davies G, et al. Lung clearance index and high-resolution computed tomography scores in primary ciliary dyskinesia. *Am J Respir Crit Care Med* 2013; **188**(5): 545-9.
126. Nyilas S, Schlegtendal A, Yammine S, Casaulta C, Latzin P, Koerner-Rettberg C. Further evidence for an association between LCI and FEV1 in patients with PCD. *Thorax* 2015; **70**(9): 896.
127. Kobbernagel HE, Green K, Ring AM, Buchvald FF, Rosthøj S, Gustafsson PM, Nielsen KG. One-year evolution and variability in multiple-breath washout indices in children and young adults with primary ciliary dyskinesia. Eur Clin Respir J. 2019 Mar 26;6(1):1591841

128. Santamaria F, Montella S, Tiddens H, et al. Structural and functional lung disease in primary ciliary dyskinesia. *Chest* 2008; **134**(2): 351-7.
129. Magnin ML, Cros P, Beydon N, et al. Longitudinal lung function and structural changes in children with primary ciliary dyskinesia. *Ped Pulmonol* 2012; **47**(8): 816-25.
130. Bhalla M, Turcios N, Aponte V, et al. Cystic fibrosis: scoring system with thin-section CT. *Radiology* 1991; **179**(3): 783-8.
131. Brody AS, Klein JS, Molina PL, Quan J, Bean JA, Wilmott RW. High-resolution computed tomography in young patients with cystic fibrosis: distribution of abnormalities and correlation with pulmonary function tests. *J Pediatr* 2004; **145**(1): 32-8.
132. Tadd K, Morgan L, Rosenow T, et al. CF derived scoring systems do not fully describe the range of structural changes seen on CT scans in PCD. *Ped Pulm* 2019; **54**(4): 471-7.
133. Montella S, Santamaria F, Salvatore M, et al. Lung disease assessment in primary ciliary dyskinesia: a comparison between chest high-field magnetic resonance imaging and high-resolution computed tomography findings. *Ital J Pediatr* 2009; **35**(1): 24.
134. Smith LJ, West N, Hughes D, et al. Imaging Lung Function Abnormalities in Primary Ciliary Dyskinesia Using Hyperpolarized Gas Ventilation MRI. *Ann Am Thorac Soc* 2018; **15**(12): 1487-90.
135. Schofield LM, Duff A, Brennan C. Airway Clearance Techniques for Primary Ciliary Dyskinesia; is the Cystic Fibrosis literature portable? *Paediatr Respir Rev. 2018; **25**:73-77*
136. Paff T, Daniels JM, Weersink EJ, Lutter R, Vonk Noordegraaf A, Haarman EG. A randomised controlled trial on the effect of inhaled hypertonic saline on quality of life in primary ciliary dyskinesia. *Eur Respir J* 2017; **49**(2).
137. Desai M, Weller PH, Spencer DA. Clinical benefit from nebulized human recombinant DNase in Kartagener's syndrome. *Ped Pulm* 1995; **20**(5): 307-8.
138. ten Berge M, Brinkhorst G, Kroon AA, de Jongste JC. DNase treatment in primary ciliary dyskinesia--assessment by nocturnal pulse oximetry. *Ped Pulm* 1999; **27**(1): 59-61.
139. El-Abiad NM, Clifton S, Nasr SZ. Long-term use of nebulized human recombinant DNase1 in two siblings with primary ciliary dyskinesia. *Respir Med* 2007; **101**(10): 2224-6.
140. Yang C, Montgomery M. Dornase alfa for cystic fibrosis. *Cochrane Database Syst Rev.* 2018; **9**: Cd001127.
141. O'Donnell AE, Barker AF, Ilowite JS, Fick RB. Treatment of idiopathic bronchiectasis with aerosolized recombinant human DNase I. rhDNase Study Group. *Chest* 1998; **113**(5): 1329-34.
142. Lucas JS, Gahleitner F, Amorim A, et al. Pulmonary exacerbations in patients with primary ciliary dyskinesia: an expert consensus definition for use in clinical trials. *ERJ Open Res 2019; **5**(1).*
143. Wijers CD, Chmiel JF, Gaston BM. Bacterial infections in patients with primary ciliary dyskinesia: Comparison with cystic fibrosis. *Chron Respir Dis 2017; **14**(4): 392-406.*
144. Kobbernagel HE, Buchvald FF, Haarman EG, et al. Study protocol, rationale and recruitment in a European multi-centre randomized controlled trial to determine the efficacy and safety of azithromycin maintenance therapy for 6 months in primary ciliary dyskinesia. *BMC Pulm Med* 2016; **16**(1): 104.
145. Shah A, Shoemark A, MacNeill SJ, et al. A longitudinal study characterising a large adult primary ciliary dyskinesia population. *Eur Respir J* 2016; **48**(2): 441-50.
146. Dell SD, Leigh MW, Lucas JS, et al. Primary Ciliary Dyskinesia: First Health-related Quality-of-Life Measures for Pediatric Patients. *Ann Am Thorac Soc* 2016; **13**(10): 1726-35.
147. Strippoli MP, Frischer T, Barbato A, et al. Management of primary ciliary dyskinesia in European children: recommendations and clinical practice. *Eur Respir J* 2012; **39**(6): 1482-91.
148. Andersen TN, Alanin MC, von Buchwald C, Nielsen LH. A longitudinal evaluation of hearing and ventilation tube insertion in patients with primary ciliary dyskinesia. *Int J Ped Otorhinolaryngology 2016; **89**: 164-8.*
149. Pruliere-Escabasse V, Coste A, Chauvin P, et al. Otologic features in children with primary ciliary dyskinesia. *Arch Otolaryngology--Head & Neck Surgery 2010; **136**(11): 1121-6.*

150. Wolter NE, Dell SD, James AL, Campisi P. Middle ear ventilation in children with primary ciliary dyskinesia. *Int J Ped Otorhinolaryngology 2012; **76**(11): 1565-8.*
151. Chhin B, Negre D, Merrot O, et al. Ciliary beating recovery in deficient human airway epithelial cells after lentivirus ex vivo gene therapy. *PLoS Genetics* 2009; **5**(3): e1000422.
152. Ostrowski LE, Yin W, Patel M, et al. Restoring ciliary function to differentiated primary ciliary dyskinesia cells with a lentiviral vector. *Gene Therapy* 2014; **21**(3): 253-61.
153. Lai M, Pifferi M, Bush A, et al. Gene editing of DNAH11 restores normal cilia motility in primary ciliary dyskinesia. *J Med Genetics 2016; **53**(4): 242-9.*
154. Bukowy-Bieryllo Z, Dabrowski M, Witt M, Zietkiewicz E. Aminoglycoside-stimulated readthrough of premature termination codons in selected genes involved in primary ciliary dyskinesia. *RNA Biology* 2016; **13**(10): 1041-50.
155. Fassad MR, Shoemark A, le Borgne P, et al. C11orf70 Mutations Disrupting the Intraflagellar Transport-Dependent Assembly of Multiple Axonemal Dyneins Cause Primary Ciliary Dyskinesia. *Am J Hum Genet 2018; **102**(5): 956-72.*
156. Antony D, Becker-Heck A, Zariwala MA, et al. Mutations in CCDC39 and CCDC40 are the major cause of primary ciliary dyskinesia with axonemal disorganization and absent inner dynein arms. *Hum Mutat* 2013; **34**(3): 462-72.
157. Horani A, Brody SL, Ferkol TW, et al. CCDC65 mutation causes primary ciliary dyskinesia with normal ultrastructure and hyperkinetic cilia. *PloS One* 2013; **8**(8): e72299.
158. Daniels ML, Leigh MW, Davis SD, et al. Founder mutation in RSPH4A identified in patients of Hispanic descent with primary ciliary dyskinesia. *Hum Mutat* 2013; **34**(10): 1352-6.